IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Arlene RAMSINGH *et al.*

Serial No.: 09/879,572

Filing Date: June 12, 2001

For: COXSACKIEVIRUS B4 EXPRESSION VECTORS AND USES THEREOF

Examiner: Donna C. Wortman

Group Art Unit: 1648

Customer No.

26694

PATENT TRADEMARK OFFICE

SECOND DECLARATION OF ARLENE I. RAMSINGH PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, the undersigned, declare as follows:

1. I am a co-inventor of the above-referenced patent application, and am familiar with its contents.

2. I am an expert in the field of molecular virology/viral immunology. I am currently a Research Scientist at the Wadsworth Center of the New York State Department of Health, in Albany, and have been at this institution for 17 years. I earned a PhD in Molecular Virology in 1983 at the University of Alberta followed by Post-doctoral training at Yale University until 1986. I am a co-author of over 25 papers in this field which were published in peer-reviewed journals. My *Curriculum Vitae* is attached.

3. I have read the Office Action mailed 27 August 2003. I understand that the Examiner has made a new rejection, citing my and my colleagues' publication, Caggana, M, Chan, P, and Ramsingh, A (1993) *J. Virol.* **67**:4797-4803 (referred to below as "Caggana") as anticipating claims 1, 3, 4, 18, and 20-26. The Examiner has also maintained the earlier rejection stating that claims 1, 3-6, 13-15, 17, 18, 20-23 and 28 are obvious in view of the patent publication of Tracy *et al.*, WO 98/39426 (referred to below as "Tracy"). I understand that The Examiner has not accepted any of the

the 8 points asserted in our previous response in support of the patentability of the claims over the Tracy reference.

4. In the sections below, I wish to make several points concerning both the cited references, including further analysis of Tracy, to help clarify for the Examiner some of the key distinctions between our invention and what these references can properly be considered to teach. My comments are made with respect to the newly amended set of claims being submitted at this time.

5. As a brief preface to my comments on the Caggana reference, I wish to emphasize the differences between Replacement vector and Insertion vector technology. For a general discussion, see, for example, Sambrook *et al.* (1989). *Molecular Cloning,* Cold Spring Harbor Laboratory Press, NY. The terms "replacement" and "insertion" originated with bacteriophage λ vectors. Because only about 60% of the viral genome is needed for the λ phage lytic cycle, the central one-third of the phage genome can be replaced by a length of foreign DNA. The term "replacement vector" referred to a λ phage whose genome included a pair of restriction enzyme sites flanking a segment of "nonessential" λ DNA that could be replaced. After digestion with the appropriate enzymes, the non-essential DNA would be replaced by the foreign DNA. The term "insertion vector" was used to refer to a phage vector with a single target site for insertion of foreign DNA which would be added to the original phage "genomic" DNA.

6. The Caggana publication cited by the Examiner described a replacement cloning strategy in which defined segments of the full-length CB4-P cDNA clone were replaced with **same size** segments from CB4-V cDNA clones. There was no net change in the coding capacity of the recombinant viruses. Hence, the newly introduced DNA simply "replaced" a stretch of "native" DNA. This contrasts with the "insertion" cloning strategy used in the present application (also described in the present application (see also, Halim *et al.* (2000a) *AIDS Res. Hum. Retrovir.* **16:**1551-1558). According to the present invention, heterologous DNA sequences (such as those encoding ovalbumin or HIV gag p24) are "inserted" into the CB4-P cloning vector. The vectors of the present invention are all "insertion vectors, recombinant viruses with "additional" DNA sequences and coding capacities that exceed that of the parental CB4-P virus. This is one basis for distinguishing the claims from Caggana.

7. Amended independent claims 1 and 18 are directed to heterologous DNA and heterologous polypeptides that are now defined more explicitly as not originating from coxsackieviruses. Even without these changes, there were grounds for the position that, contrary to the Examiner's assertion, the CB4-P/CB4-V chimeras of Caggana do not include what are commonly accepted as "heterologous" sequences. However, in view of the amendments, even that reasoning is unnecessary to point out the clear distinction between the claimed virions that include heterologous DNA encoding a heterologous polypeptide that is by definition foreign to any coxsackievirus, and certainly to the particular strains/serotypes of CVB4 that are described by the claims.

8. Understanding the similarities and differences between Coxsackieviruses B3 and B4, helps one appreciate the rather significant differences between the Tracy disclosure and the present invention. The group B coxsackieviruses ("CVB") belong to the enterovirus genus of the family *Picornaviridae*. The picornaviruses share a common genome organization (Racaniello, VR, *Picornaviridae: The viruses and their replication.* In: Fields Virology, 4th Ed, Lippincott Williams & Wilkins, Philadelphia, PA, 2001). Group B includes six serotypes designated B1-B6. A sequence comparison of B3 and B4 shows a high degree of similarity (88% of the predicted amino acid sequence of the polyprotein; Jenkins, O *et al.* (1987) *J Gen Virol* 68:1835-1848)

9. ***Differences between CVB-3 and CVB-4 Serotypes***

Despite certain similarities, which the Examiner correctly pointed out (at least with respect to genome organization), there are crucial differences between these serotypes which have not been given due attention. These relate not so much to the issue of *attenuation* raised in our previous response as to immunological differences.

A. Antigenicity

Infections with CVB induce production of antibodies of two different "levels" of specificity: (1) Group B-specific antibodies recognize both CVB-3 and CVB-4, and (2) serotype-specific antibodies which distinguish CVB-4 from CVB-3 (Gauntt, 1997, *Curr Top Microbiol Immunol* 223:259-282.). An important distinction between these two classes of antibodies, that reflect the immunogenic constructs of the present invention, is that serotype-specific antibodies are virus-neutralizing whereas group B-specific antibodies are not. Thus, infection by or immunization with a

CVB3 virus or vaccine does not lead to a protective antibody response against infection with CVB4. Similarly, infection by or immunization with a CVB4 virus or vaccine does not protect against infection with CVB3. Thus, there would be no expectation that an immunogenic virion or viral vector construct to be used, for example as a vaccine, based on one virus would result in a similar level of immunity as a vaccine based on the other virus.

My laboratory (Halim SS *et al.* (2000b) *Virology* 269:86-94) identified a linear B cell epitope of CVB4 and showed that this epitope contained a neutralization antigenic site. The neutralization antigenic site appeared to be serotype-specific since the peptide that included the B cell epitope did not inhibit the neutralizing activity of sera from mice infected with CVB3 (CVB3/0, obtained from Tracy).

B. Molecular determinants of viral virulence (see previous response)

CVB3 is recognized as the agent of cardiac disease while CVB4 is generally associated with pancreatic disease. The molecular determinants of virulence for CVB3-induced cardiac disease and CVB4-induced pancreatic disease are very different, and depend not just on serotype but also on strain. Studying the H3 variant of CVB3, Knowlton *et al.* (1996). *J.Virol.* 70, 7811-18; of record) showed that virulence was attenuated by a mutation in an external loop of VP2. For clinical isolates of CVB3, the 5' UTR was identified as the determinant of cardiovirulence (Dunn, JJ *et al.*, 2000, *J.Virol.* 74:4787-94; of record). For CVB4, my laboratory identified two determinants of pancreatic disease in the VP1 and VP4 capsid proteins (Caggana *et al.*, *supra*; Ramsingh & Collins (1995) *Virology 69*:7278-81). This issue was discussed in the previous Response.

10. *Brief Review of Antigen Processing, Role of MHC Class I and Class II Pathways, and Epitopes Recognized by Different T Cell Subsets*[1]

To reiterate some basic immunological concepts, adaptive immune responses consist of B and T cell responses. B cells produce antibodies whereas T cells are needed to control intracellular pathogens and to activate B cell responses to most antigens. Two major types of T cells, T helper (Th) and cytotoxic T lymphocytes (CTL) recognize peptides bound to two different classes of MHC proteins. The requirements for generating a CTL response differ from those for a Th and B cell response. For induction of a CTL response, an antigenic protein within a cell is degraded to peptides

1 See, for example, Janeway, CA *et al.*, (2001). Immunobiology: *The Immune System in Health and Disease*." Garland

which are transported into the endoplasmic reticulum, processed through the MHC class I pathway and presented to T cells. CTLs generated in response to such presentation then recognize peptides within the context of MHC class I proteins on a target cell surface. T helper (Th) cells are induced after an exogenous antigen (*e.g.*, a virion) has been ingested by antigen-presenting cells (APCs), where "Th epitopes" are loaded on to MHC class II proteins and transported to the cell surface where they are recognized in the context of the class II proteins by Th cells. A key challenge in vaccine development is to be able to present epitopes to the immune system so that desired Th, CTL and B cell responses are induced.

11. Cloning strategy for engineered immunogenic virions

The cloning strategy to generate recombinant viruses influences the type of immune response generated against the foreign sequence. Said otherwise, the purpose to which the vector is to be put, *e.g.*, serving as a vaccine construct for inducing an antibody response, influences the cloning strategy.

The following remarks about the Tracy reference are made not in a vacuum, but in consideration of all the relevant published work emanating Tracy's laboratory, with which I am completely familiar. The Tracy reference, which employs CVB3[2] describe a strategy in which the heterologous protein is never expressed as part of the virion structure. Rather it is expressed intracellularly when the infecting virus's genes are transcribed, translated and processed. In part this is because the expressed heterologous protein is cleaved away from the polyprotein by a viral protease, so it never reach the stage of virion particle processing and packaging for release. An "antigenic" polypeptide expressed according to the Tracy method can, at best, serve as a CTL epitope. Any virion produced according to Tracy is only "recombinant" with respect to its RNA genome, as there are no recombinant proteins (comprising a heterologous sequence) in these virions. The recombinant virions of our invention carry the heterologous polypeptide sequence fused to a capsid protein.

Tracy states at page 16, lines, 10-13, of the cited patent publication, that:

> The heterologous nucleic acid sequence carried by the coxsackievirus vector of the invention can encode any gene product, including RNA of any kind, peptides and proteins.

Publishing, New York; also referred to below as "Janeway textbook"

2 and this is true for related poliovirus vector studies described, for example, in U.S. Pat. 5,965,124 to Feinberg *et al.*)

and further states at page 15, lines 20-27:

> ... a heterologous DNA segment can be inserted in the CVB3 genome in one of several locations, e.g., between a coding sequence for a capsid protein and a coding sequence for viral protease, or at the start of the genome's open reading frame, in such a manner that the heterologous DNA comprises a translation start codon and a 3' sequence recognized by a viral protease.

This approach is based on inserting foreign sequences at junctional sites in the viral genome. The first site referred to above, where Tracy describes insertion of a heterologous sequence is the junction, P1/P2.[3] The second site disclosed is the 5'UTR/P1 junction. It is noteworthy that an insert at the second site specifically requires a recognition sequence for a viral protease (and indeed, Tracy's group added such an additional site to their recombinant viruses!). The purpose for these requirements is several-fold. First, Tracy wants to avoid the heterologous protein becoming part of, or fused to, a structural viral protein that ends up in the virion itself as it may contort and inactivate the virus (the precise opposite of the embodiment of our invention in claim 1). By limiting the inserts to junctional sites, further noting the importance of protease recognition sites in the protein, Tracy's approach assures that the foreign sequences will be processed intracellularly and released. If such peptides are intended as immunogens, they will of necessity be limited to processing by the MHC class I pathway, which is only useful for *potential* CTL epitopes. (Obviously, the heterologous amino acid sequence must include epitopes that are "appropriate" for the intended host's T cell receptor repertoire!). Both these outcomes are in accord with Tracy's desire -- that the heterologous proteins only "exist" briefly inside the cell during a certain phase of the virus's life cycle. It should be evident that this is **totally distinct** from the present claims which require the heterologous polypeptide sequence to be part of the actual virion, specifically, as a fusion with a viral capsid protein. This is achieved by inserting the heterologous nucleic acid into the capsid genes, as disclosed in the specification. This cannot happen with the Tracy approach.

Along the same line, it is noteworthy that Tracy's publications (cited in Footnote 3) both assert that insertion at the 5'UTR/P1 junction results in genetically unstable recombinants. Our results with CVB-4 as disclosed in the specification dispute this (see also Halim, SS *et al.* (2000a) *supra*).

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3 See, also other publications from Tracy's laboratory: Chapman, N *et al.* (2000). J.Virol. 74, 7952-7962.; Hofling, K *et al.* (2000) J.Virol. 74, 4570-4578)

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

2/27/04 Arlene I. Ramsingh

Date Arlene I. Ramsingh

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CURRENT POSITION: Research Scientist,
Wadsworth Center, David Axelrod Institute for Public Health
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EMPLOYMENT

1986-present: Research Scientist (Grades II, III and, presently, IV)
Wadsworth Center for Laboratories and Research
New York State Department of Health
Albany, New York

1983-1986: Post-doctoral fellow
Department of Human Genetics
Yale University School of Medicine, New Haven, CT
(mentor: Dr. Sherman Weissman)

EDUCATION

University of Alberta, Canada	Ph.D.	1983	Molecular Virology
University of Toronto, Canada	M.Sc.	1978	Virology
University of Toronto, Canada	B.Sc.	1976	Microbiology

GRANTS

Active

National Institutes of Health 2002-2004
T cell immunity to HIV using recombinant enteroviruses

Previous

American Heart Association 1998-2001
Molecular pathogenesis of virus-induced myocarditis

National Institutes of Health 1992-1997
Pancreatitis induced by coxsackievirus B4

American Heart Association 1991-1994
Pathogenesis of coxsackievirus B4-induced myocarditis

Diabetes Research and Education Foundation 1990-1991
Molecular characterization of viruses implicated in type 1 diabetes

American Diabetes Association 1987-1989
Analysis of the molecular events underlying the development of insulin- dependent diabetes mellitus (IDDM)

PROFESSIONAL SOCIETIES

- American Diabetes Association, American Heart Association, American Association for the Advancement of Science, American Society of Microbiology, American Society of Virology

ADDITIONAL PROFESSIONAL ACTIVITIES

Review Board: National Institutes of Health Special Emphasis Panel (July '02)

Ad hoc reviewer: Journal of Virology, Archives of Virology, International Journal of Experimental Diabetes Research, Virology

Invited seminar speaker

Cornell University Medical School, New York NY
Albany Medical School, Albany NY
Connaught Biotechnology Research Institute, Toronto, Canada
SUNY, Albany NY
University of Vermont, Burlington VT
Renssalaer Polytechnic Institute, Troy NY
University of Nebraska Medical Center, Omaha, NE
First Northern Immunology Meeting, Garnet Hill, NY
Second Northern Immunology Meeting, Garnet Hill, NY

TEACHING AND STUDENT ADVISING

Graduate: Served as mentor for three doctoral students; supervised numerous graduate students during laboratory rotations and served on numerous thesis advisory committees

Undergraduate: Advised large number of undergraduates

SERVICE

- Served on various committees for the ***School of Public Health, State University of New York at Albany*** and for the ***Wadsworth Center for Laboratories and Research r***

PUBLICATIONS

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Halim, S., Ostrowski, S., Lee, W., **Ramsingh, A.I.** Immunogenicity of a foreign peptide expressed within a capsid protein of an attenuated coxsackievirus. Vaccine 19:958-965, 2001.

Potvin, D.M., Metzger, D.W., Lee, W.T., Collins, D.N., **Ramsingh, A.I.** Exogenous interleukin-12 protects against lethal infection with coxsackievirus B4. J. Virol. 77:8272-8279, 2003

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Ramsingh, A.I., Chapman, N., Tracy, S. Coxsackieviruses and diabetes. BioEssays 19(9):793-800, 1997.

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Huber, S., and **Ramsingh, A.I.** Coxsackieviruses and inflammatory pancreatic disease. Viral Immunology (in preparation)